Iranian Journal of Veterinary Science and Technology

Received: 2022- Mar- 09
Accepted after revision: 2022- Jun- 18
Published online: 2022- Jun- 30

RESEARCH ARTICLE

DOI: 10.22067/ijvst.2022.75347.1117

The effect of Artemisinin on the Pentylentetrazole-induced seizures during the estrous cycle and GABA interaction in mice

Mahsa Barkhordarian[a], Jahangir Kaboutari[a], Morteza Zendehdel[b], Saeid Habibian Dehkordi[a]

[a] Department of Basic Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.
[b] Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

ABSTRACT

Catamenial epilepsy may involve 10 to 70% of women with epilepsy in which, seizures are exacerbated by the menstrual cycle. Artemisinin is a herbal compound with widespread modern and traditional medical indications. Because of GABAergic interaction, this study was designed to study the antiepileptic effects of Artemisinin during the estrus cycle. A total of 360 adult female mice were placed in 10 groups: control, solvent (ethanol 10ml/kg), Artemisinin (75&150 mg/kg), Bicuculline (2mg/kg), Bicuculline (2mg/kg) + Atremisinin (75&150 mg/kg), Saclofen (2mg/kg), Saclofen (2mg/kg) + Atremisinin (75&150 mg/kg), each with four subgroups (proestrus, estrus, metestrus and diestrus) (n=9). One week after acclimatization, estrous synchronization and phase determination was achieved. Acute epilepsy was induced by intraperitoneal (i.p) injection of 80 mg/kg of Pentylentetrazole (PTZ), 30 minutes after i.p injection of Artemisinin and ethanol. Initiation time of myoclonic seizures (ITMS), initiation time of tonic-clonic seizures (ITTS), seizure duration (SD), and mortality rate (MR) were recorded for 30 minutes. Data were displayed as mean ± SD and evaluated using one-way ANOVA followed by Tukey–Kramer multiple comparison post hoc tests ($p < 0.05$). Artemisinin significantly decreased epilepsy incidence, duration, and mortality rate, in parallel to increasing ITMS and ITTS in a dose-dependent manner which were more prominent during the luteal phase. Co-administration of Biccucluline significantly inhibited antiepileptic effects of Artemisinin, while Saclofen did not have such an inhibitory interaction. It seems that increased neurosteroid metabolites and GABAA receptors, neural hyperpolarization following GABA interaction, and anti-inflammatory and anti-oxidative properties which decrease neuroinflammation and neural excitability can participate in the antiepileptic effects of Artemisinin.

Keywords

Artemisinin, catamenial epilepsy, estrous cycle, GABA

Number of Figures: 3
Number of Tables: 2
Number of References:: 22
Number of Pages: 7

Abbreviations

AEDs: Antiepileptic drugs
AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
AP: Allopregnanolone
ART: Artemisinin
BIC: Bicucculine
GABA: Gama aminobutyric acid

Corresponding author: Jahangir Kaboutari
Email: kaboutari-j@sku.ac.ir
Tel: +90(38)3424-2427

Introduction

Epilepsy is one of the most common and classical CNS diseases, defined as unpredictable repetitive seizures, which may involve 0.5-1% of the people in industrial countries. Epilepsy affects life quality and social relations of the patients. In 10 to 70% of women with epilepsy, particularly those with focal or generalized types, a cyclic model of seizure exacerbation aligns with the menstrual cycle, especially around the perimenstrual or the periovulatory period has been seen. Thus, based on the Greek word katomenios or monthly, it has been called catamenial epilepsy. Sex steroids and their metabolites play a critical role in seizure initiation and propagation via contributing to the development and organization of the CNS and neural excitability since progesterone exerts anticonvulsant while estrogens exert proconvulsant characteristics [1,2,3,4,5]. Despite numerous antiepileptic drugs (AEDs) which can control epilepsy in about 60% of patients, catamenial epilepsy remains a drug-resistant form [6]. Because of the side effects of AEDs such as hepatotoxicity, an increased desire for natural remedies and safer treatments such as herbal ones are unavoidable. Artemisinin is an endoperoxide sesquiterpene lactone isolated from Artemisia annua that has wide-ranging folkloric and contemporary remedial indications e.g. anti-malarial, anti-fungal, anti-bacterial, anti-leishmania, anti-coccidian, anti-diabetic, anti-spasmodic, anti-oxidative, anti-inflammatory, analgesic, and wound healing [7]. Artemisia species contain considerable sources of flavonoids, which can act as positive allosteric modulators of the GABAA receptor, so display anxiolytic, sedative, and antinociceptive actions [7]. Artemisinin interacts with the Gephyrin part of the GABAA receptor complex, activates it, increases GABA signaling, and inhibits nociceptive signaling in the dorsal root ganglia leading to analgesia [8,9]. Artesunate can protect against maximal electroshock and PTZ-induced seizures in mice [10]. Basal levels of progesterone in the brain can alter GABA receptor density throughout the estrous cycle [11]. Based on these findings and fluctuating levels of steroid hormones and their metabolites along the estrous cycle, this study was planned to investigate the effect of Artemisinin on the PTZ-induced seizures during the estrous cycle and the possible role of the GABAergic system.

Abbreviations-Cont'd

ITMS: Initiation time of myoclonic seizures
i.p.: intraperitoneal
ITTS: Initiation time of tonic-clonic seizure
MR: mortality rate
NMDA: N-Methyl-D-aspartate
PTZ: Pentylentetrazole
SAC: Saclofen
SD: seizure duration

Results

Seizure duration

Artemisinin significantly decreased seizure duration through the estrous cycle, dose-dependently ($p < 0.05$). There was no significant difference between Saclofen, Bicucciline, Bisucculine plus Artemisinin and control ($p > 0.05$). While the difference between the Artemisinin and Artemisinin-Saclofen combination was not significant ($p > 0.05$), Saclofen + Aartemisinin significantly lowered seizure duration than that in the control group ($p < 0.05$). In all treatment groups, seizure duration was significantly lower in the luteal phase than in the follicular phase ($p < 0.05$) (Table 1).

Mortality rate

Artemisinin administration, significantly and dose-dependently decreased the mortality rate in comparison to that in the control group, all along the estrous cycle ($p < 0.05$). There was no significant difference between groups that received Saclofen, Bicucciline, Bisucculine plus Artemisinin, and the control group ($p > 0.05$), but Salcofen + Artemisinin co-administration significantly decreased the mortality rate in comparison to the control ($p < 0.05$). In all groups, the mortality rate was significantly lower during the luteal phase than in the follicular phase ($p < 0.05$) (Table 2).

ITMS

Artemisinin significantly and dose-dependently increased ITMS, ($p < 0.05$), which in all groups, it was significantly higher during the luteal phase than in the follicular phase ($p < 0.05$). There was no significant difference between groups that received Saclofen, Bicucciline, Bisucculine plus Artemisinin, and the control group ($p > 0.05$), but Salcofen + Artemisinin co-administration significantly decreased ITMS in comparison to the control group ($p > 0.05$) during the entire estrous cycle (Figure 1).

ITTS

ITTS was significantly increased by Artemisinin in a dose-dependent way. In all groups, it was significantly higher during the luteal phase than the follicular phase ($p < 0.05$). In all phases of the estrous cycle, there was no significant difference between groups that received Saclofen, Bicucciline, Bisucculine plus Artemisinin, and the control group ($p > 0.05$), but Salcofen + Artemisinin co-administration significantly decreased ITTS in comparison to the control group (Figure 2).

Discussion

To the authors' knowledge, this is the first study about the effect of Artemisinin on PTZ-induced seizures during the estrous cycle and GABAergic interaction. Decreasing seizure incidence, duration, and mortality rate in addition to increasing ITTS and ITMS indicated antiepileptic properties of Artemisinin, that through the luteal phase, it was markedly more than the follicular phase. These antiepileptic properties can be mediated by the GABAA recep-

Table 1.
Effects of Bicuculline (BIC; 2mg/kg), Saclofen (2 mg/kg), Artemisinin (ART; 75 and 150 mg/kg) and combination of BIC (2 mg/kg) and Saclofen (2 mg/kg) with ART (75 and 150 mg/kg) on seizure duration (SD) (sec) during various phases of the estrous cycle.

Group/Estrous Cycle	Proestrous	Estrous	Metestrous	Diestrous
Control	771 ± 41^{a}	752 ± 53^{a}	507 ± 41^{a*}	513 ± 39^{a*}
Vehicle	753 ± 37^{a}	731 ± 34^{a}	531 ± 41^{a*}	524 ± 36^{a*}
BIC (2mg/kg)	801 ± 72^{a}	793 ± 64^{a}	578 ± 38^{a*}	584 ± 53^{a*}
Saclofen (2 mg/kg)	741 ± 63^{a}	712 ± 65^{a}	491 ± 36^{a*}	497 ± 31^{a*}
ART (75 mg/kg)	570 ± 43b	527 ± 39b	401 ± 37^{b*}	366 ± 35^{b*}
ART (150 mg/kg)	374 ± 33c	392 ± 46c	237± 25c *	218 ± 25^{c*}
BIC (2 mg/kg) + ART (75 mg/kg)	731 ± 34^{a}	722 ± 64^{a}	536 ± 34^{a*}	505 ± 35^{a*}
BIC (2 mg/kg) + ART (150 mg/kg)	701 ± 58^{a}	729 ± 71^{a}	536 ± 56^{a*}	572 ± 51^{a*}
Saclofen (2 mg/kg)+ ART (75 mg/kg)	552 ± 34^{b}	518 ± 28^{b}	418 ± 31^{b*}	356 ± 27^{b*}
Saclofen (2 mg/kg)+ ART (150 mg/kg)	361 ± 47^{c}	406 ± 54^{c}	208 ± 45^{c*}	215 ± 49^{c*}

Different letters (a, b, or c) in each column indicate a significant difference ($p < 0.05$) between various treatments in each phase of the estrous cycle. Asterisk (*) indicates a significant difference for each phase of the estrous cycle Vs the control group ($p < 0.05$). Data are presented as mean ± SEM.

Table 2.
Effect of Bicuculline (BIC; 2 mg/kg), Saclofen (2 mg/kg), Artemisinin (ART; 75 and 150 mg/kg) and combination of BIC (2 mg/kg) and Saclofen (2 mg/kg) with ART (75 and 150 mg/kg) on the mortality rate (MR) of seizures (%) during various phases of the estrous cycle.

Group Estrous Cycle	Proestrous	Estrous	Metestrous	Diestrous
Control	33.3^{a}	33.3^{a}	16.6^{*a}	16.6^{*a}
Vehicle	33.3^{a}	33.3^{a}	16.6^{*a}	16.6^{*a}
BIC (2 mg/kg)	33.3^{a}	33.3^{a}	16.6^{*a}	16.6^{*a}
Saclofen (2 mg/kg)	33.3^{a}	33.3^{a}	16.6^{*a}	16.6^{*a}
ART (75 mg/kg)	16.6^{b}	33.3^{a}	16.6^{*a}	16.6^{*a}
ART (150 mg/kg)	0^{c}	0^{b}	0^{b}	0^{b}
BIC (2 mg/kg) + ART (75 mg/kg)	33.3^{a}	33.3a	16.6^{*a}	16.6^{*a}
BIC (2 mg/kg) + ART (150 mg/kg)	33.3^{a}	33.3a	16.6^{*a}	16.6^{*a}
Saclofen (2 mg/kg) + ART (75 mg/kg)	16.6^{b}	33.3^{a}	16.6^{*a}	16.6^{*a}
Saclofen (2 mg/kg) + ART (150 mg/kg)	0^{c}	0^{b}	0^{b}	0^{b}

Different letters (a, b, or c) in each column indicate significant differences at the $p < 0.05$ level between various treatments in each phase of the estrous cycle. Asterisk (*) indicates a significant difference for each phase of the estrous cycle Vs the control group ($p < 0.05$).
Data are presented as mean ± SEM.

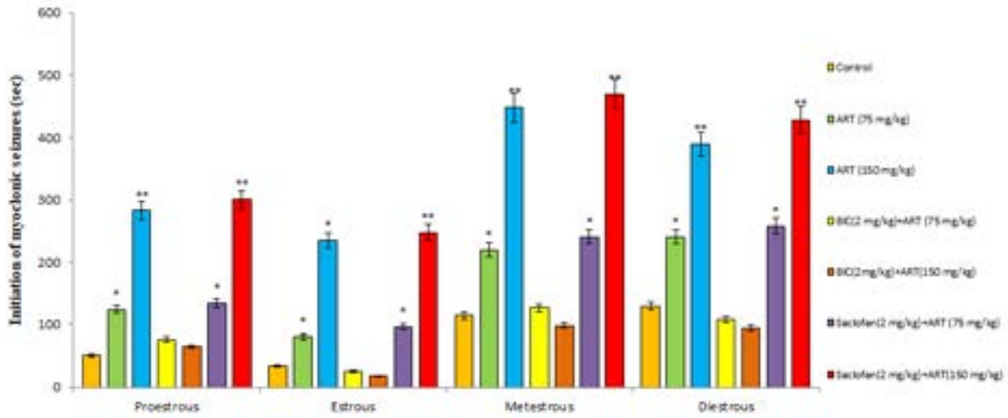

Figure 1.
Effects of Artemisinin (ART; 75&150 mg/kg), Bicuculline (BIC; 2mg/kg), Saclofen (SAC, 2 mg/kg) and combination of BIC (2 mg/kg), SAC (2 mg/kg), and (ART; 75 & 150 mg/kg) on the initiation time of myoclonic seizures (ITMS) (sec) during the estrous cycle. *Asterisks indicate a significant difference in each phase of the estrous cycle compared with that in the control group ($p < 0.05$). Data are presented as mean ± SEM.

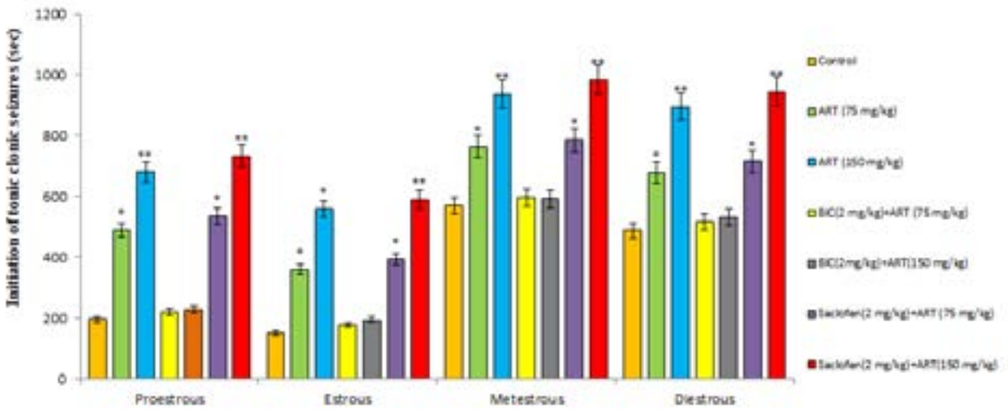

Figure 2.
Effects of Artemisinin (ART; 75&150 mg/kg), Bicuculline (BIC; 2mg/kg), Saclofen (2; mg/kg) and combination of BIC (2 mg/kg), SAC (2 mg/kg), and ART (75 & 150 mg/kg) on the initiation time of tonic-clonic seizures (ITTS) (sec) during the estrous cycle. *Asterisks indicate a significant difference in each phase of the estrous cycle compared with that in the control group ($p < 0.05$). Data are presented as mean ± SEM.

tors since GABAA receptors blocked by Bicuculline inhibited antiepileptic effects of Artemisinin while GABAB receptors blocked by Salcofen did not interfere with these effects.

Periodical variation in the circulating level of estrogens and progesterone through the estrous cycle may engage in the pathophysiology of catamenial epilepsy. Sex steroids and their metabolites govern the synthesis, discharge, and transport of different neurotransmitters and exert significant function in the pathophysiology of seizures. Estrogens are proconvulsant by repressing GABA synthesis and amplitude of GABA-mediated inhibitory postsynaptic current while augmenting the dendritic spines and NMDA synapses on the hippocampus CA1 pyramidal neurons, increasing glutamatergic transmission which enhances neural excitability [1,2,5]. The direct interrelationship between estrogen level and estrogen/progesterone proportion with seizure vulnerability indicates maximum vulnerability and exacerbation during the premenstrual and periovulatory periods. Perovulatory aggravation may be owing to an estrogen upsurge in the mid cycle, nearly, uncontested by the progesterone before the luteal phase, while, during the premenstrual phase, a lower level of progesterone than estrogen can assist periovulatory seizure exacerbation [1,2,4,5]. Meanwhile, there is a negative correlation between progesterone level and catamenial epilepsy, so that, rapid fall in the progesterone level just before, during menstruation, and then after, is correlated with an increase in catamenial seizures. Progesterone potentiates the inhibitory action of adenosine, decreases neural firing, epileptiform discharges, estrogen receptors, and counteracts estrogen proconvulsant effects via metabolization to the neurosteroid metabolite and enhancement of GABA-mediated inhibition [5,6,12,13]. Neurosteroids have been discovered primarily in some brain divisions such as the hippocampus and neocortex, adjusting various neurotransmitter signaling systems, including GABAA receptors, membrane progesterone receptors, NMDA receptors, and L or T type calcium channels, and protect against different models of seizure. Neurosteroids with a 3α-hydroxy, 5α-reduced structure such as Allopregnanolone (AP) are positive allosteric modulators of GABAA receptors that increase cell surface expression of GABAA receptors, meanwhile, they are negative allosteric modulators of NMDA and AMPA excitatory receptors [4,12,13,14,15,16,17]. Additionally, at higher concentrations, they can activate GABAA receptors directly, and increase the frequency and opening time of chloride channels, therefore, rapidly diminishing neural excitability [4]. GABAA receptors are the most important and abundant inhibitory receptors in the CNS, which induce neural hyperpolarization and dampen neural excitability [7]. Suppressing the neuroprotective effects of AP by GABAA antagonists proves the GABAergic mechanism of action of the neurosteroids [16]. In the female rats, AP shows higher antiepileptic properties during diestrous than estrous, probably due to the progesterone-induced enhancement of GABAA receptor and neurosteroid metabolite production [1,2]. Artemisinin inhibits morphological changes of hippocampus neurons, induces glutamic acid decarboxylase enzyme, and increases GABA level, which protects against sodium penicillin-induced seizures [15]. It interacts with the Gephyrin segment, triggers GABAA receptors, and enhances GABA singling which can be blocked with GABAA antagonists. Gephyrin is a part of the GABAA receptor that exerts an essential role in the distribution of GABAA and glycine receptors to the cell membrane, therefore playing a cardinal function in their inhibitory action [8,9]. Artemisinin interaction with GABAA receptors results in CNS depression and analgesia [7]. Artesunate restricts the seizure spread in the maximal electroshock (MES)-induced seizure by inhibiting Na+ channels and/or glutamatergic excitation via NMDA receptors, so may be helpful in the management of generalized tonic-clonic and partial seizures [10]. Progesterone regulates and increases GABA receptor density in the cerebral cortex, hippocampus, and hypothalamus during the luteal phase, where they act presynaptically to increase GABAergic while modulating glutamatergic release, also inducing G protein-mediated late inhibitory postsynaptic potential [11,18,19]. Bicuculline is a competitive antagonist of the ionotropic GABAA receptors in the hippocampal or cortical neurons, and blocks inhibitory action on the target neurons leading to epilepsy, while Saclofen is a competitive antagonist for the metabotropic GABAB receptors [20]. In this study, Bicuculline inhibited the antiepileptic actions of Artemisinin on the SD, MR, ITTS, and ITMS, while administration of Saclofen did not show such an inhibitory effect, so, it can be concluded that the anti-epileptic effect of Artemisinin is mediated by the GABAA receptors.

There is a direct relation between ovarian progesterone and neurosteroid level so that, due to common metabolic pathways, the enzyme-inducing property of Artemisinin can increase AP concentration and therefore enhances the anticonvulsant effect during the luteal phase. It is in line with a previous report about higher antiepileptic effects of enzyme-inducing AEDs such as carbamazepine, during the luteal phase of the estrus cycle [21].

Artemisinin showed a significant antiepileptic effect against PTZ-induced seizures along the estrous cycle, especially during the luteal phase. It seems that induction of progestin metabolism, increasing neurosteroids level, interaction with GABAA receptors, and neural hyperpolarization in addition to anti-inflammatory and anti-oxidative features decrease neuroinflammation and neural excitability that can lead to the antiepileptic effects of Artemisinin.

Materials & Methods

Animals and experimental design

360 adult female albino N-MRI mice weighing 25-30 grams were received from the University animal house and kept under established conditions, according to the European community protocols for the protection of animals used for scientific purposes with freely available fresh, clean water, and chow pellets. Experiments were carried out following the guidelines for the care and use of laboratory animals to investigate experimental pain in animals [22], approved by The University Research Ethics Committee (97GRN1M1904). After one week of familiarization, animals were split coincidentally into 10 groups

(n = 9) and 4 trials. At first, by examination of the vaginal smears, sexual puberty was determined. The estrous cycle phase was decided based on the chief cell of the vaginal smears, and mice with two regular estrous cycles were chosen. Then, estrous synchronization was set [3,21]. Experiment 1 was achieved to study the effect of Artemisinin on the PTZ-induced seizures during the proestrus phase. The seizure was induced by i.p. injection of 80 mg/kg of PTZ (Sigma-Aldrich, USA) 30 minutes after i.p. administration of 75 and 150 mg/kg Artemisinin (Alexis Biochemicals, USA) and 10ml/kg of ethanol as solvent (Merck, Germany) (Table 3). Then, animals were observed for 30 minutes and antiepileptic parameters including ITTS, ITMS, MR, and SD were charted. Other experiments were done similarly to evaluate the effects of Bicuculline, GABAA antagonist (BIC; 2mg/kg, Sigma-Aldrich, USA), Saclofen, GABAB antagonist (SAC, 2 mg/kg, Sigma-Aldrich, USA), and a combination of BIC (2 mg/kg) and SAC (2 mg/kg) with ART (75 & 150 mg/kg) during estrous, metestrous, and diestrous phases. For prevention of the likely effect of circadian rhythm on seizure susceptibility, all experiments were fulfilled between 9 am to 3 pm [3,21]. Finally, the animals were euthanized by i.p administration of a high dose of Sodium thiopental (Novartis, Switzerland).

Statistical analysis

Data are summarized as mean ± SEM and analyzed by one-way analysis of variances (ANOVA) followed by Tukey–Kramer multiple comparison post hoc tests ($p < 0.05$).

Table 3.
Treatment procedure in Experiment 1.

Group	1st injection	2nd injection*
Control	*Normal saline*	PTZ (80 mg/kg)
ART (75 mg/kg)	*ART (75 mg/kg)*	PTZ (80 mg/kg)
ART (150 mg/kg)	*ART (150 mg/kg)*	PTZ (80 mg/kg)

*30 min after the first injection; Pentylenetetrazol (PTZ); Artemisinin(ART)

Authors' Contributions

J.K.: Study design, final manuscript preparation; M.Z.: Statistical analysis; S.H.D.: primary manuscript preparation; M.B.: animal study and data collection.

Acknowledgements

The authors appreciate the Deputy of Research of Shahrekord University for financial support.

Competing Interests

The authors declare that they have no conflict of interest.

References

1-Herzog AG. Catamenial epilepsy: definition, prevalence pathophysiology and treatment. Seizure. 2008; 17(2):151-159.

2-Herzog AG. Catamenial epilepsy: Update on prevalence, pathophysiology and treatment from the findings of the NIH Progesterone Treatment Trial. Seizure. 2015; 28:18-25.

3- Kaboutari J, Zendehdel M, Habibian S, Azimi M, Shaker M, Karimi B. The antiepileptic effect of sodium valproate during different phases of the estrous cycle in PTZ-induced seizures in rats. J Physiol Biochem. 2012; 68(2):155-161.

4-Reddy DS. Neuroendocrine aspects of catamenial epilepsy. Horm behave. 2013; 63(2):254-266.

5-Verrotti A, D'Egidio C, Agostinelli S, Verrotti C, Pavone P. Diagnosis and management of catamenial seizures: a review. Int J women's health.2012; 4:535–541.

6- Dhir A. Curcumin in epilepsy disorders. Phytother Res. 2018;32(10):1865-1875.

7- Dehkordi FM, Kaboutari J, Zendehdel M, Javdani M. The Antinocicptive effect of Artemisinin on the inflammatory pain and role of GABAergic and opioidergic systems. Korean J Pain.2019; 32 (3): 160-167.

8-Li J, Casteels T, Frogne T, Ingvorsen C, Honoré C, Courtney M, et al. Artemisinin targets GABAA receptor signaling and impair α cell identity. Cell.2017; 168: 86-100.

9-Liu HK. Artemisinin GABA signaling and cell reprogramming: when an old drug meets modern medicine. Sci Bul.2017; 62: 386-87.

10-Sanjana K, Shyamjith M, Deepa B, Rao SN, Pai PG. Effect of artesunate on maximal electroshock and pentylenetetrazole-induced seizures in albino mice. Int J Green Pharm. 2012;6(1)63-66.

11-Xin W, Omkaram G, Chase MC, Doodipala SR. Estrous Cycle Regulation of Extrasynaptic δ-Containing GABAA Receptor-Mediated Tonic Inhibition and Limbic Epileptogenesis. J Pharmacol Exp Ther.2013; 346(1):146–160.

12-Reddy DS, Woodrad R. Ganaxolone: a prospective overview. Drugs fut.2004; 29: 227-242.

13-Reddy DS. The role of neurosteroids in the pathophysiology and treatment of catamenial epilepsy. Epilepsy Res.2009; 85:1-30.

14-Borowicz K, Czuczwar S, Piskorska B, Banach M. Neuroprotective actions of neurosteroids. Front endocrinol.2011;2:50-60.

15-Xin HE, Lu H, Yang LU, Mengmeng DOU, et al. The Protective Effects of Artemisinin on Penicillin Sodium-Induced Epileptic Seizures in Mice. Lat Am J Pharm.2016; 35(4):1241-1247.

16-Mendell, AL, MacLusky NJ. Neurosteroid metabolites of testosterone and progesterone differentially inhibit ERK phosphorylation induced by amyloid β in SH-SY5Y cells and primary cortical neurons. Brain Res.2018; 1686: 83-93.

17-Joshi S, Kapur J. Neurosteroid regulation of GABAA receptors: a role in catamenial epilepsy. Brain Res.2019; 1703:31-40.

20- Johnston, Graham AR. Advantages of an antagonist: bicuculline and other GABA antagonists. Brit J Pharmacol.2013; 169 (2): 328–336.

18-Al-Dahan MI, Tehrani MHJ, Thalmann RH. Regulation of γ-aminobutyric acid B (GABAB) receptors in cerebral cortex during the estrous cycle. Brain Res.1994; 640: 33-39.

19-Al-Dahan MI, Thalman RH. Progesterone regulates gamma-aminobutyric acid B (GABAB) receptors in the neocortex of female rats. Brain Res.1996; 727: 40-48.

21-Zendehdel M, Kaboutari J, Ghadimi D, Hassanpour S. The antiepileptic effect of ghrelin during different phases of the estrous cycle in PTZ-induced seizures in rat. Int J Pept Res Ther.2014; 20(4): 511-517.

22-Zimmermann M (1983). Ethical guidelines for investigations of experimental pain in conscious animals. Pain.1983; 16: 109-10.

How to cite this article
Barkhordarian M, Kaboutari J, Zendehdel M, Habibian Dehkordi S.TThe effect of Artemisinin on the Pentylentetrazole induced seizures during the estrous cycle and GABA interaction in mice. Iran J Vet Sci Technol. 14(2): 55-61.
DOI: https://doi.org/ 10.22067/ijvst.2022.75347.1117
URL:https://ijvst.um.ac.ir/article_42528.html